

GCSF-03-2004-B.ST25
SEQUENCE LISTING

<110> Sun, Lee-Hwei K
Sun, Bill
Sun, Cecily R

<120> Fc fusion proteins of human granulocyte colony-stimulating factor with increased biological activities

<130> 03SUN2001-B

<140> to be assigned

<141> 2004-03-15

<150> US 09/968362

<151> 2001-10-01

<160> 28

<170> PatentIn version 3.1

<210> 1

<211> 30

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<213> Artificial Sequence

<220>

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<223> PCR primer

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27

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<223> PCR primer

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gagcgcaaat gttgtgtcga

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<210> 4

<211> 28

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<213> Artificial Sequence

<220>

<223> PCR primer

<400> 4

ggaattctca tttacccgga gacagggga

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<213> Artificial Sequence

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<223> PCR primer

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ttgtgtcga

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gagtccaaat atggtccccc a

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<211> 28

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<213> Artificial Sequence

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<223> PCR primer

<400> 9

ggaattctca ttaccacaga gacagga

28

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<211> 21

<212> DNA

<213> Artificial Sequence

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<223> PCR primer

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21

<210> 11

<211> 60

<212> DNA

<213> Artificial Sequence

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<223> PCR primer

<400> 11

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<211> 70

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 <213> Artificial Sequence
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 <223> PCR primer
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<210> 16

<211> 70

<212> DNA

<213> Artificial Sequence

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<223> PCR primer

<400> 16
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 cacatgcccc 70

<210> 17

<211> 1368

<212> DNA

<213> Artificial Sequence

<220>

<223> hG-CSF-L-VFc gamma2 (Figure 2A)

<400> 17
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 gccagctccc tgccccagag cttcctgctc aagtgccttag agcaagtgag gaagatccag 180
 ggcgatggcg cagcgtccca ggagaagctg tgtgccacct acaagctgtg ccaccccgag 240
 gagctgggtg tgctcggaca ctctctgggc atccccctggg ctcccctgag cagctgcccc 300
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 caggggctcc tgcaggccct ggaagggatc tcccccgagt tgggtccac cttggacaca 420
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 cgcgttctac gccaccttgc ccagcccga tccggtggcg gttccggtgg aggcggaagc 660
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<210> 18

<211> 448

<212> PRT

<213> Artificial Sequence

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<223> hG-CSF-L-VFc gamma2 with a 30-amino acid leader peptide (Figure 2A)

<400> 18

Met Ala Gly Pro Ala Thr Gln Ser Pro Met Lys Leu Met Ala Leu Gln
1 5 10 15

Leu Leu Leu Trp His Ser Ala Leu Trp Thr Val Gln Glu Ala Thr Pro
20 25 30

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu
35 40 45

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys
50 55 60

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu
65 70 75 80

Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser
85 90 95

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu
100 105 110

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Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu
 115 120 125
 Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala
 130 135 140
 Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu
 145 150 155 160
 Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg
 165 170 175
 Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu
 180 185 190
 Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Gly Ser Gly Gly
 195 200 205
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Arg Lys Cys
 210 215 220
 Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
 225 230 235 240
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
 245 250 255
 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
 260 265 270
 Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 275 280 285
 Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val
 290 295 300
 Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 305 310 315 320
 Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Ser Ile Glu Lys Thr
 325 330 335
 Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
 340 345 350
 Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
 355 360 365

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Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp
385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
405 410 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 19

<211> 1371

<212> DNA

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma4 (Figure 2B)

<400> 19

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gccagctccc tgccccagag cttcctgctc aagtgttag agcaagttag gaagatccag	180
ggcgtatggcg cagcgtcca ggagaagctg tgtgccacct acaagctgtg ccaccccag	240
gagctggtgc tgctcggaca ctctctgggc atccccctggg ctcccctgag cagctgcccc	300
agccaggccc tgcagctggc aggctgcttg agccaactcc atagcggcct tttcctctac	360
caggggctcc tgcaggccct ggaagggatc tccccgagt tgggtccac cttggacaca	420
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atggcccctg ccctgcagcc caccagggt gccatgccgg ccttcgcctc tgctttccag	540
cgccgggcag gaggggtcct agttgcctcc catctgcaga gcttcctgga ggtgtcgtag	600
cgcgttctac gccaccttgc ccagcccga tccgggtggcg gttccggtgg aggcggaagc	660
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<210> 20

<211> 449

<212> PRT

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma4 with a 30-amino acid leader peptide (Figure 2 B)

<400> 20

Met Ala Gly Pro Ala Thr Gln Ser Pro Met Lys Leu Met Ala Leu Gln
1 5 10 15

Leu Leu Leu Trp His Ser Ala Leu Trp Thr Val Gln Glu Ala Thr Pro
20 25 30

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu
35 40 45

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys
50 55 60

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu
65 70 75 80

Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser
85 90 95

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu
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105

110

Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu
 115 120 125

Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala
 130 135 140

Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu
 145 150 155 160

Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg
 165 170 175

Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu
 180 185 190

Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Gly Ser Gly Gly
 195 200 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ser Lys Tyr
 210 215 220

Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala Gly Gly Pro
 225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp
 260 265 270

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val
 290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
 325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 340 345 350

Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys
 405 410 415

Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
 435 440 445

Lys

<210> 21

<211> 1365

<212> DNA

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma1 (Figure 2C)

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aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1320
tacacgcaga agagcctctc cctgtctccg ggtaaatgag aattc 1365

<210> 22

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<223> hG-CSF-L-vFc gamma1 with a 30-amino acid leader peptide (Figure 2 C)

<400> 22

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1 5 10 15

Leu Leu Leu Trp His Ser Ala Leu Trp Thr Val Gln Glu Ala Thr Pro
20 25 30

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu
35 40 45

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys
50 55 60

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu
65 70 75 80

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Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser
85 90 95

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu
100 105 110

Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu
115 120 125

Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala
130 135 140

Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu
145 150 155 160

Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg
165 170 175

Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu
180 185 190

Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Gly Ser Gly Gly
195 200 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Val Ala Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile
325 330 335

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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 23

<211> 16

<212> PRT

<213> Artificial sequence, 16-amino acid peptide linker

<400> 23

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1 5 10 15

<210> 24

<211> 15

<212> PRT

<213> Human IgG1 hinge sequence

<400> 24

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

<210> 25

<211> 10

<212> PRT

<213> Truncated human IgG1 hinge sequence

<400> 25

Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10

<210> 26

<211> 232

<212> PRT

<213> Human IgG1 Fc with native hinge, CH2 and CH3 domains

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Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

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Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 27

<211> 228

<212> PRT

<213> Human IgG2 Fc with native hinge, CH2 and CH3 domains

<400> 27

Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val
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Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65 70 75 80

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn
85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro
100 105 110

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Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln
115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
165 170 175

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
210 215 220

Ser Pro Gly Lys
225

<210> 28

<211> 229

<212> PRT

<213> Human IgG4 Fc with native hinge, CH2 and CH3 domains

<400> 28

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
1 5 10 15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35 40 45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
100 105 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
210 215 220

Leu Ser Leu Gly Lys
225